OPEN ACCESS Freely available online

Cell & Developmental Biology

Research Article

Shoot Regeneration Capability and Partial Self-Incompatibility of a *Taraxacum kok-saghyz* Germplasm

Grisel Ponciano, Kumiko Johnson, Grace Q Chen*

Department of Agriculture, Western Regional Research Center, Agricultural Research Service, Albany, USA

ABSTRACT

Rubber dandelion *Taraxacum kok-saghyz* (Tk) has been developed as an alternative crop to diversify natural rubber production. Germplasm utilization and biotechnology are potential approaches to developing commercially viable Tk crops. We surveyed individuals from a Tk germplasm (W6-35169) to assess their overall plant morphology, *in vitro* plantlets' regeneration capacity and self-incompatibility. Plants exhibited different leaf and root morphology. Using combinations of plant hormones, naphthalene acetic acid and benzylaminopurine, 83 calli were induced from 23 seedlings with the capacity of 42% to 79%. Most calli developed unviable shoots such as leafy-like structures or albino shoots. Few calli produced significant numbers of viable shoots ranged from 26-187 per callus. We hand-pollinated 31 plants and found two individuals capable of producing selfed seeds. We identified plant #12 which was competent in producing shoots and selfed seeds. This is the first report for describing a novel approach for discovering an individual particularly useful in implementing a genetic approach for basic and applied research to improve a Tk crop.

Keywords: *Taraxacum kok-saghyz*; *In vitro* shoot generation; Self-compatibility; Benzylaminopurine; Calli

INTRODUCTION

The perennial rubber dandelion *Taraxacum kok-saghyz* (Tk) (also known as Russian or Kazakh dandelion), is a rubber-producing plant native to the Tien Shan mountains' valleys in Kazakhstan. Tk is currently being developed as an alternative commercial source of natural rubber in North America, Asia and Europe where cultivation of the tropical tree *Hevea brasiliensis*, the conventional source of natural rubber, is not suitable. Tk produces rubber mainly in the root. Due to its Self-Incompatibility (SI) in seed production, Tk exhibits high variability in important traits such as root morphology and rubber content, therefore it has not been fully domesticated yet. An alternative approach to Tk improvement is genetic engineering. Published literature is available for Tk genetic transformation and a few on the closely related species *Taraxacum brevicorniculatum* and *Taraxacum platycarpum*. The transgenic plants generated from some of these experiments were not shown to transmit transgenes to the next generation of seeds and in most cases the transformation efficiency was not reported [1].

In this study, we conducted a survey of a Tk germplasm for its general plant morphology as well as capacity in generation of calli, shoots and roots under tissue culture conditions. Also, we explored the potential of overcoming self-incompatibility in Tk by hand-pollination. We aim to identify individuals allowing efficient *in vitro* shoot regeneration as well as producing viable seeds through selfing. Our results provide useful guidance for further evaluation of the entire germplasm collection with the goal of identifying the best candidates for Tk crop development [2].

MATERIALS AND METHODS

Plant material, growth condition and hand-pollination

Diploid Tk seeds (accession No. KAZ08-014, ID W6-35169) were obtained from the United States Department of Agriculture Plant Germplasm System (NPGS, https://www.ars-grin.gov/npgs/). This Tk accession was collected from the wild and

Correspondence to: Grace Q Chen, Department of Agriculture, Western Regional Research Center, Agricultural Research Service, Albany, USA; E-mail: grace.chen@usda.gov

Received: 09-Dec-2024, Manuscript No. CDB-24-35728; **Editor assigned:** 12-Dec-2024, PreQC No. CDB-24-35728 (PQ); **Reviewed:** 26-Dec-2024, QC No. CDB-24-35728; **Revised:** 07-Jan-2026, Manuscript No. CDB-24-35728 (R); **Published:** 14-Jan-2026, DOI: 10.35248/2168-9296.26.15.421

Citation: Ponciano G, Johnson K, Chen GQ (2026) Shoot Regeneration Capability and Partial Self-Incompatibility of a *Taraxacum kok-saghyz* Germplasm. Cell Dev Biol. 15:421.

Copyright: © 2026 Ponciano G, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

maintained in a research field protected with insect proof screen cage. Insects or bees were introduced into the cage to allow for cross-pollination and set seeds. In our greenhouse, Tk plants were grown and maintained as described. For hand-pollination, two flower heads (capitula) from the same plant were rubbed together to make selfed seeds. Open-ended 6 × 9 cm lightweight nets were used to isolate the pollinated heads [3].

Tissue culture propagation

Tk seeds were surface-sterilized in 5% (v/v) sodium hypochlorite and 0.1% (v/v) Tween-20 solution for 20 min followed by five rinses in sterile deionized water. The seeds were cold treated at 4°C for 4 days and germinated and grown aseptically on germination medium containing half-strength MS medium plus 0.5% sucrose and 0.6% agar, pH 5.8 in growth chamber at a day/night cycle (16/8 h, 100 μmol/m^2/s) at 24°C (day) and 22°C (night). Media formulations for each tissue culture stage are all based on half-strength MS media plus 2% sucrose and 0.6% agar, pH 5.8, supplemented with vitamins and the following hormones: a) For callus induction medium R1: 0.1 mg/L of Naphthalene Acetic Acid (NAA)+1 mg/L of Benzylaminopurine (BA); callus induction medium R2: 0.1 mg/L of NAA+2 mg/L of BA; callus induction medium R3: 0.2 mg/L of NAA+1 mg/L of BA; and callus induction medium R4: 0.2 mg/L of NAA+2 mg/L of BA; b) For shoot induction medium, 2 mg/L (C3) or 1 mg/L of BA (C4); and c) For root induction medium, 1 mg/L 3-Indolebutyric Acid (IBA). Callus and shoot induction media was refreshed through subculture explants once every two weeks [4].

RESULTS AND DISCUSSION

Seed germination and plant morphology

After three weeks in germination media, 65% of sterilized seeds (31/48) successfully germinated. Five weeks later, all plantlets developed three to four leaves with the oldest ones of 4-5 cm in length. Differences in leaf morphology were also observed. Some of the plants have an oblanceolate leaf shape (Figure 1A) with an entire margin and some have serrated leaf margins (Figure 1B) [5]. Root morphologies were documented when the plants reached adult stage. Some plants had a long tap root and few lateral roots (Figure 1C), some had branched roots (Figure 1D) and others had a short tap root that branched to few smaller roots (Figure 1E). The morphological differences of leaves and root system architecture among individual Tk plants are indicative of genetic heterogeneity within the same accession. Similar leaf and root morphology has been reported [6].

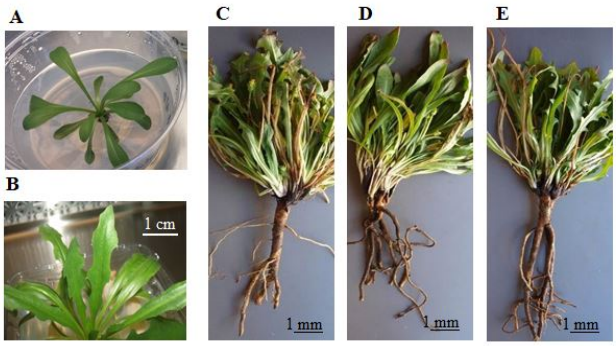

Figure 1: Leaf and root morphology of Tk W6-35169 germplasm representatives. Five-week-old seedlings showing oblanceolate leaves (A) or serrated leaves (B). Mature plants showing tap (C), branched (D) and combination tap and branched root (E).

Callus induction

To derive explants from the 31 seedlings, leaves were the tissue of choice. Leaves were harvested and cut in half along the middle vein, and subsequently, each half was cut into ~5-10 mm sections (Figure 2A). To induce callus, three leaf sections (or explants) of each seedling were maintained in four types of callus induction media R1-R4. Seedlings after a leaf was taken for callus induction were transferred to soil for the maturation phase of growth. We designed to cut through the central middle vein of a leaf and presumed more vascular bundles to be exposed to the media [7].

The vascular bundle contains xylem, phloem and a layer of cambium cells which are undifferentiated primitive cells and possess high proliferating capability. Indeed, we observed that after two weeks in callus induction media, some leaf sections had developed callus about the size of a pin-head and the location of the callus was at the edge of the leaf section where the middle vein would be (Figure 2A). After 25 days of callus induction, callus grew to the size of 5-10 mm diameter with some of them developed shoot-like structures (Figure 2B). We note that some cultures were discontinued due to various reasons, including leaves from two seedlings never developed callus in any of these four media, leaves from two seedlings had contamination and calli from four seedlings eventually turned necrotic [8].

We scored the numbers of callus after 33 days of induction and the results are shown in Table 1. All four types of media enabled callus induction, with R4 (media supplemented with the highest hormone concentration, 0.2 mg/L NAA+2 mg/L BA) developing the most calli, followed by R3, R2 and R1. There was no noticeable morphological difference among calli generated from any of these four media. Our media design scheme was four combinations by two doses of NAA (0.1 mg/L or 0.2 mg/L) × two doses of BA (1 mg/L or 2 mg/L), which are in similar combinations of NAA and BA reported for induction of callus and shoot in Tk. Interestingly, a recent study reported Tk shoot induction and regeneration from leaf explants without callus formation in the presence of BA concentration at 1.5 mg/L. It is worth noting that the Tk germplasm of that study is different from the one reported here [9].

Table 1: Callus induction media screening in Tk W6-35169.

Callus induction media	Number of explants (number of seedlings)	Number of explants developing callus	Percentage explants developed callus
R1 (0.1 mg/L NAA+1 mg/L BA)	24 (8)	10	42%
R2 (0.1 mg/L NAA+2 mg/L BA)	42 (14)	24	57%
R3 (0.2 mg/L NAA+1 mg/L BA)	39 (13)	23	59%
R4 (0.2 mg/L NAA+2 mg/L BA)	33 (11)	26	79%

Note: NAA: Naphthalene Acetic Acid; BA: Benzylaminopurine

Shoot and root induction

To evaluate shoot regeneration capacity, a total of 83 calli derived from 23 seedlings were divided into half and each half was cultured in shoot induction medium C3 or C4 containing 2 mg/L or 1 mg/L BA, respectively. After 3 weeks in C3 or C4, majority calli produced unviable shoots. These unviable shoots had either shoot/leaf-like structures (Figure 2B) or were albino shoots (Figure 2C), both of which ceased growth. We found some calli generated from #1, #12 and #20 seedlings produced viable shoots (Figure 2D). The total numbers of viable shoots were counted by adding up all viable shoots derived from the same callus during 2-6 weeks of subcultures until the callus exhausted in producing more shoot tips. Overall, C4 medium generated more shoots (240 in total) than C3 (22 in total). Among the three individuals, #12 produced the most shoots (187 in total) followed by #20 (49 in total) and #1 (26 in total). Due to the limited numbers of calli competent in generating viable shoots, the numbers of total shoots per callus represent an exploratory overview of shoot regeneration capacity. As we traced callus and viable shoot induction back to individuals, the limited plants (3 out of 23) capable of producing viable shoots indicated a high heterogeneity in a Tk accession. Genetic diversity is also reported in differential rubber contents of individuals within or among Tk accessions and the rubber content is positively correlated with the levels of rubber synthesis gene expression. The wide range of shoot production capacity (0 to118 per callus) indicated that there were different cellular organization regions on the callus. It is known that callus is not a mass of uniformly dividing cells and only the cells that undergo rapid division and reorganization to meristematic tissue will give rise to shoots. The Tk accession W6-35169, KAZ-8-014, used in this study exhibited high variability in responding to tissue culture conditions, such as callus and shoot induction, which are likely genotype-dependent. This accession provides potential materials for studying the mechanisms of callus-mediated shoot regeneration in Tk. Shoots were then transferred to root induction media. After one month, all shoots grew bigger and developed green leaves and roots. Plantlets were then transferred to media without hormonal supplement for continued subculture for 1.5 months (Figure 2E).

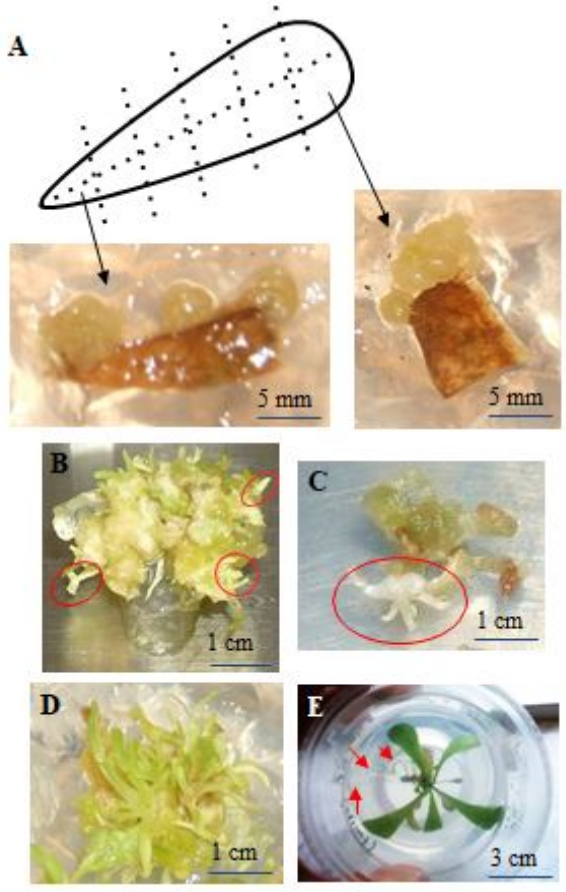

Figure 2: *In vitro* callus, shoot and root development from Tk W6-35169 leaf sections. (A) Calli formed from the edge of leaf middle vein. (B) Callus having shoot-like structures indicated by red circles. (C) An albino shoot indicated by a red circle. (D) Viable shoots. (E) Roots indicated by red arrows.

Identification of self-compatible individuals

Tk (*Asteraceae*) is a Self-Incompatible (SI) outcrossing species. Although the Tk accession used in this study were maintained and reproduced among a small group of plants, we have observed different leaf and root morphology and wide variation in callus and shoot regeneration, which suggested high heterogeneity of the Tk population. SI is a mechanism adopted

by certain species including Tk for retaining genetic diversity, but it hinders inbreeding efforts for developing desirable agronomic traits (e.g., root morphology and rubber content in Tk) for crop improvement. Also, when a transgenic Tk is created, it is difficult to obtain a homozygous transgenic line, and thus complicates the process of transgene evaluation and maintenance in progeny. It is reported that when manually self-pollinated, about 10% *Asteraceae* species are Partially Self-Incompatible (PSI) and within a species 10-15% of the population are Self-Compatible (SC). To test if PSI exists in the Tk accession, we manually self-pollinated the 31 individuals in a greenhouse. We found that two (#10 and #12) out of the 31 plants are capable of producing viable seeds. The seed germination rate was 18.7% (± 4.1% SD) and 24.7% (± 5.0% SD) for #10 and #12, respectively. The viable seeds produced by #10 and #12 indicated that Tk accession W6-35169, KAZ08-014, is PSI with preliminary SC rate at 6.5%.

The morphological and gene expression aspects of pollination processes in Tk have been explored. Microscopy analysis indicated that in Tk, SI is characterized by the arrest of pollen and limited pollen tube growth with no penetration through the stigma, whereas cross competitive pollination was characterized by pollen germination and penetration of the stigma and style. Transcriptomic data reveals three candidate genes, *LRX4*, *TUBB* and *XTH33*, encoding leucine-rich repeat extensin-like protein 4, β-tubulin and hydrolase 33, respectively, that are highly expressed and may drive pollen tube growth in Tk during competitive pollination. From evolution point of view, SI in *Asteraceae* is an ancient breeding system, PSI occurs due to loss of S-locus function or by disrupting. Mechanism underlying the PSI remained to be investigated in this Tk accession.

CONCLUSION

Tissue culture propagation from leaf explants of USDA Tk W6-35169, KAZ08-014 germplasm is possible despite its inherited heterogeneity. Additionally, some individuals of this accession are self-compatible and this characteristic in combination with tissue culture propagation capability makes it a good candidate for crop improvement through genetic engineering and/or breeding efforts.

ACKNOWLEDGEMENTS

The authors thank Dr. Barbara Hellier for providing Tk W6-35169 germplasm. USDA is an equal opportunity provider and employer. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

FUNDING STATEMENT

This work was funded by U.S. Department of Agriculture-Agricultural Research Service in-house CRIS project# 2030-21410-022-000D and USDA headquarter-funded research associate.

AUTHOR CONTRIBUTIONS

The authors confirm contribution to the paper as follows: G. Ponciano and G. Chen designed the experiment and equally contributed writing the manuscript; G. Ponciano performed all plant tissue culture; K. Johnson performed pollination experiments.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to report regarding the present study.

REFERENCES

1. Buranov AU, Elmuradov BJ. Extraction and characterization of latex and natural rubber from rubber-bearing plants. J Agr Food Chem. 2010;58(2):734-743.
2. van Beilen JB, Poirier Y. Guayule and Russian dandelion as alternative sources of natural rubber. Crit Rev Biotechnol. 2007;27(4):217-231.
3. Volis S, Uteulin K, Mills D. Russian dandelion (*Taraxacum kok-saghyz*): One more example of overcollecting in the past? J Appl Bot Food Qual. 2009;83(1):60-63.
4. Collins-Silva J, Nural AT, Skaggs A, Scott D, Hathwaik U, Woolsey R, et al. Altered levels of the *Taraxacum kok-saghyz* (Russian dandelion) small rubber particle protein, TkSRPP3, result in qualitative and quantitative changes in rubber metabolism. Phytochemistry. 2012;79:46-56.
5. Ganesh I, Choi SC, Bae SW, Park JC, Ryu SB. Heterologous activation of the Hevea PEP16 promoter in the rubber-producing laticiferous tissues of *Taraxacum kok-saghyz*. Sci Rep. 2020;10(1):10844.
6. Iaffaldano B, Zhang Y, Cornish K. CRISPR/Cas9 genome editing of rubber producing dandelion *Taraxacum kok-saghyz* using *Agrobacterium rhizogenes* without selection. Ind Crop Prod. 2016;89:356-362.
7. Knyazev A, Kuluev B, Mikhaylova E, Yasybaeva G, Chemeris A. Aseptic germination and *Agrobacterium rhizogenes*-mediated transformation of *Taraxacum kok-saghyz* Rodin. Plant Root. 2017;11:64-69.
8. Lankitus D, Zhang Y, Ariyaratne M, Barker DJ, McNulty SL, Amstutz N, et al. *Agrobacterium rhizogenes*-induced altered morphology and physiology in rubber dandelion after genetic transformation. HortScience. 2023;148:21-28.
9. Niephaus E, Muller B, van Deenen N, Lassowskat I, Bonin M, Finkemeier I, et al. Uncovering mechanisms of rubber biosynthesis in *Taraxacumsssss kok-saghyz*-role of cis-prenyltransferase-like 1 protein. Plant J. 2019;100:591-609.